# DATA EVALUATION RECORD
# FRESHWATER SEDIMENT *Chironomus riparius* EMERGENCE TEST

1. **CHEMICAL:** Trifluralin PC Code: 036101

2. **TEST MATERIAL:** Trifluralin Technical Purity: 96.3%

3. **CITATION:**

Authors: Knoch, M.
Title: Assessment of Side Effects of Trifluralin Technical on the Larvae of the Midge, *Chironomus riparius* with the Laboratory Test Method.
Study Completion Date: November 11, 1996
Laboratory: Arbeitsgemeinschaft
GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH
D-75223 Niefern-Öschelbronn, Germany
Sponsor: Dow Agrosciences LLC
9330 Zionsville Road
Indianapolis, IN 46268
Laboratory Report ID: 96015/01-ASCr
MRID No.: 478070-13

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

**Signature:** Christie E. Padova **Date:** 11/04/09

**APPROVED BY:** Teri S. Myers, Senior Scientist, Cambridge Environmental Inc.

**Signature:** Teri S Myers **Date:** 12/08/09

5. **APPROVED BY:** Christine Hartless, OPP/EFED/ERB 1

**Signature:** 5-7-10 **Date:** 5/7/10

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Chironomus riparius*
Age of Test Organism: 1st instar larvae, 2 days post-hatch
Definitive Test Duration: 28 days
Study Method: Static with aeration
Type of Concentrations: Nominal overlying water (associated TWAs were not applicable)

## 7. CONCLUSIONS:

Results Synopsis: This study is classified as Supplemental and can be used in risk characterization. It should not be used in risk estimation as there were concerns regarding the actual exposure concentrations. Some reasons this study should not be used for risk estimation:

- concentration in pore water was not measured
- concentrations in sediment were reported as mg/vessel and it was not possible to convert those values to mg/kg-dry wt of sediment
- trifluralin was detected on the film over the vessels, indicating material volatilized out of the water
- measured concentrations in overlying water did not increase consistently as nominal concentrations increased (*e.g.*, in the nominal concentrations of 1.0 and 2.0 mg/L, the measured concentrations were 0.107 and 0.058 mg/L, respectively). There was uncertainty regarding the actual exposure concentrations in the study vessels.

Emergence Percentage (nominal concentrations)
NOAEC: 2.0 mg ai/L
LOAEC: 4.0 mg ai/L
$EC_{50}$: 6.9 mg ai/L 95% C.I.: (4.6 to 10 mg ai/L)

Development Rate (nominal concentrations)
NOAEC: 0.25 mg ai/L
LOAEC: 0.5 mg ai/L
$IC_{50}$: >8.0 mg ai/L

Assessment endpoints: emergence rate and development rate (combined genders)
Most sensitive endpoint based on NOAEC: development rate

## 8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: It was reported that the study followed the BBA Guideline: Effects of plant protection products on the development of sediment-dwelling larvae of Chironomus riparius in a water sediment system (Streloke and Köpp, 1995) and OECD Guideline No. 207: Earthworm, Acute Toxicity Test, and does not fulfill any current U.S. EPA data requirement.

C. Reparability: N/A

**9. MAJOR GUIDELINE DEVIATIONS (from OECD Guideline 219):**

1. The environmental conditions maintained during culturing and the health of the in-house culture was not reported.
2. The TOC and moisture content of the artificial sediment were not reported.
3. The sediment to overlying water depth ratio was *ca.* 1:9, exceeded the maximum recommended ratio of 1:4.
4. Water hardness and ammonia levels were not monitored during the study.
5. Analysis of pore water for trifluralin concentrations was not performed.

**10. SUBMISSION PURPOSE:** Litigation/Endangered Species

**11. MATERIALS AND METHODS**

**Stability of Compound Under Test Conditions:** To assess the behavior of trifluralin in the test system, samples of overlying water collected from the 1.0 and 8.0 mg/L levels (biological test systems) were analyzed on Days 0, 3, 7, 28, and samples of sediment collected from the 1.0 and 8.0 mg/L levels (surrogate test systems) were analyzed on Days 0, 7, and 28. Data reported for the overlying water from surrogate test systems were not included in this discussion (see Reviewer's Comments section).

Trifluralin dissipates from the water phase rapidly. At Day 0 (2 hours following application), maximum concentrations in overlying water were 41 and 3.4% of nominal levels at the 1.0 and 8.0 mg/L levels, respectively. The lower recovery at the 8.0 mg/L level was an effect of the low aqueous solubility of trifluralin and its tendency to aggregate at concentrations above its solubility. By Day 7, trifluralin was undetectable in overlying water at both concentration levels.

In sediment, concentrations of trifluralin were 19.8 and 58.9% of nominal levels on Day 0 at the 1.0 and 8.0 mg/L levels, respectively, indicating that at concentrations above the solubility, nearly 60% of the applied is transferred to the sediment. It could not be determined whether transfer to the sediment or volatilization from solution was responsible for the loss. It was noted that the color of the parafilm cage (not further described) turned to yellow during the test, and apparently an analysis of the cage was performed (methods not reported). At the 1.0 and 8.0 mg/L levels on Day 7, concentrations of trifluralin in sediment were 15.6 and 62.7% of the nominally applied, respectively, and concentrations in/on the cage were 19.0 and 2.9% of the nominally applied, respectively. On Day 28, trifluralin was not detected in sediment and accounted for 2.1% of the applied at the 1.0 mg/L level, and accounted for 41.5 and 2.8% of the nominally applied in the sediment and cage, respectively, at the 8.0 mg/L level.

**Physicochemical properties of trifluralin.**

| Parameter | Values | Comments |
|---|---|---|
| Water solubility at 20°C | <1.0 mg/L | Temp. not reported |
| Vapor pressure | Not reported | |
| UV adsorption | Not reported | |
| pKa | Not reported | |
| Kow | Not reported | |

*OECD requires water solubility, stability in water and light, $pK_a$, $P_{ow}$, and vapor pressure of the test compound.*

**A. Test Organisms/Acclimation**

| Guideline Criteria | Reported Information |
|---|---|
| **Species**<br>*Chironomus riparius* | *Chironomus riparius* |
| **Source** | In-house cultures. |
| **Culture Conditions**<br>A reproduction and oviposit chamber should consist of an adult area, sufficiently large to allow swarming (minimum 30 x 30 x 30 cm), and an oviposit area. Crystallizing dishes or larger containers with a thin layer of quartz sand (5 to 10 mm) or Kieselgur (thin layer to a few mm) spread over the bottom and containing suitable water to a depth of several cm are suitable as an oviposit area. Environmental conditions: temperature 20±2°C; 16:8 hours light:dark (intensity ca. 1000 lux); air humidity ca. 60% | Chironomid larvae were reared in glass dishes containing a thin layer of fine quartz sand and 7- to 8-cm of Elendt medium M4 (dilution water). The larval rearing vessels were maintained within a suitable cage to maintain emerged adults. Environmental conditions were not reported. |

| Guideline Criteria | Reported Information |
|---|---|
| **Egg Mass Acclimation Period**<br>Four to five days before test initiation freshly laid egg masses should be taken from cultures and maintained separately in culture medium, temperature change should not exceed 2°C per day. | Four days before test initiation, freshly-laid eggs masses were collected from the cultures and deposited into small vessels in culture medium (temperature not reported). |
| **Age of Test Larvae**<br>First instar (1 to 4 days post-hatch with confirmation) | 1st instar, 2 days post-hatch |
| **Food**<br>Green algae (e.g., *Scenedesmus subspicatus, Chlorella vulgaris*) or flaked fish food as a ground powder, suspension, or filtrate | Chironomus larvae were fed every second or third day (*ad libitum*) with ground Tetra Min® fish flake food at *ca.* 25 mg per vessel per day. |
| **Health of parent culture stock**<br>Were parent chironomids in good health during the culture period? | Not reported |

**B. Test System**

| Guideline Criteria | Reported Information |
|---|---|
| **Type of Test System**<br>Static (static-renewal or flow-through of overlying water is evaluated on a chemical-specific basis). Distilled or deionized water may be added to overlying water once daily as needed to maintain volume. | Static with aeration. The water level was marked (presumably so that evaporative losses could be replaced). |
| **Test Materials** | Identity: trifluralin, technical<br>Common name: trifluralin<br>Physical description: orange solid<br>Lot No.: RMM 1915<br>Purity: 96.3% (w:w); analyzed<br>Storage: room temperature in the dark |

| Guideline Criteria | Reported Information |
|---|---|
| **Stock Solutions** | A primary stock solution was prepared in acetone, and dosing solutions were prepared from the primary stock.<br><br>Dosing stock solutions were applied just below the water surface using a Hamilton pipette, and were gently stirred with a glass rod after addition. |
| **Test Water**<br>Soft reconstituted water or water from a natural source is preferred. Dechlorinated tap water may be used if the test organism will survive in it for the duration of the culturing and testing without showing signs of stress. | Elendt M4 Medium was prepared using analytical-grade salts and de-ionized water. A detailed composition was provided. The artificial medium had a $Ca^{2+}/Mg^{2+}$ proportion of 4/1, a $Na^{+}/K^{+}$ proportion of 10/1, an acid capacity of 0.8 mmol/L, a total hardness of 14.5°dH, and a pH of 7.5 ± 0.3. |
| **Test Sediment**<br>Formulated (reconstituted, artificial, or synthetic) sediment is recommended. Content of sediment by dry weight: 5% peat (dry) (pH 5.5-6.0) or alpha-cellulose, 75% quartz sand (>50% in size range of 50-200 microns), 20% kaolinite clay (kaolinite content ca. 30%), $CaCO_3$ 0.05-0.1%). Moisture content 30-50%, TOC 2% (±0.5%) and pH 6.5 - 7.5. Natural sediment can be used if it is fully characterized, unpolluted, and free of organisms that might compete with or consume chironomids. (If solvent other than water will be used, sand content of artificial sediment is adjusted accordingly.) | Formulated (artificial) sediment was prepared (according to OECD Guideline No. 207; 1984) as follows (dry weight basis):<br>70% industrial sand (>50% between 50 and 200 µm), 20% kaolin clay (kaolinite content >30%), and 10% sphagnum peat (pH 5.5 to 6.0, with no visible plant remains, air-dried and finely ground).<br><br>The final pH was adjusted by the addition of *ca.* 1% calcium carbonate. The dry constituents were mixed thoroughly using an electric mixer. De-ionized water was added to moisten the sediment prior to use.<br><br>TOC: not reported<br>Moisture content: not reported<br>pH: 6.0 ± 0.5 |

| Guideline Criteria | Reported Information |
|---|---|
| **Sediment Conditioning**<br>Artificial sediment: 7 days in flowing dilution water prior to test initiation, chambers may be aerated | Test vessels (sediment:water) were prepared 5 to 7 days prior to study initiation (Day 0) and acclimated under test conditions. |
| **Introduction of Test Organisms**<br>Twenty-four hours prior to test initiation aeration of chambers is stopped and organisms are added to the chambers. Aeration should not resume for at least 24 hours. At test initiation, the test substance is spiked into the overlying water column. | One day prior to test initiation (i.e., Day -1), midge larvae were impartially added to each replicate test vessel. Aeration was stopped while the animals were added and for the following 24 hours. |
| **Solvents**<br>If used, minimal (i.e., ≤0.1 ml/l) and same concentration in all treatments. Suitable solvents are acetone, ethanol, methanol, elthylene glycol monoethyl ether, ethylene glycol dimethyl ether, dimethylformamide or triethylene glycol. (OECD guidelines also allows use of dispersants: Cremophor RH40, Tween 80, methycellulose 0.01%, and HCO-40) | Acetone<br>0.1 mg/L test solution |
| **Water Temperature**<br>20°C ± 2°C (Should not deviate between vessels by more than 1°C.) | 19.4 to 21.5°C |
| **pH**<br>Sediment: 7.0 ± 0.5<br>Interstitial Water:<br>Overlying Water: 6.0 to 9.0<br>(Should not vary by more than 1 unit during test) | Sediment: 6.0 ± 0.5 (at preparation)<br>Interstitial Water: Not determined<br>Overlying Water: overall range of 7.48 to 10.58, with daily averages increasing from 7.93 on Day -1 to 9.99 on Day 27 |
| **TOC**<br>Sediment: 2 ± 0.5%<br>Overlying Water: 2 mg/L | Sediment: Not determined<br>Overlying Water: Not determined |

| Guideline Criteria | Reported Information |
|---|---|
| **Ammonia**<br>Interstitial Water:<br>Overlying Water: | Interstitial Water: Not determined<br>Overlying Water: Not determined |
| **Total Water Hardness**<br>200 mg/L as $CaCO_3$ (prefer 160 to 180 mg/L as $CaCO_3$) | Not determined |
| **Dissolved Oxygen**<br>60% air saturation value throughout test | 8.0 to 12.9 mg/L (>60% saturation) |
| **Aeration**<br>Aeration (ca. one bubble/sec) is allowed except for when larvae are being added and for at least 24 hours after introduction of test organisms to a test chamber. If one test chamber is aerated all test chambers must be treated the same. | Continuously at a rate of 1-2 bubbles/sec, except for during and approximately 24 hours following the addition of the larvae. |
| **Test Vessels or Compartments**<br>1. Material: Glass, No. 316 stainless steel, teflon or perfluorocarbon plastics<br>2. Size: Sediment depth of 1.5- 3 cm and the depth ratio of sediment to water should be ca. 1:4, must not be >1:4; 600 ml beaker with 8 cm diameter | Material: glass beakers<br><br>Size: 2 L (13-cm diameter), containing a 2.0-cm layer of sediment (270 g wet weight) and a 15- to 20-cm layer of overlying water (1600 mL). The height ratio was *ca.* 1:9 sediment to overlying water. |
| **Covers**<br>Test vessels should be covered with a glass plate. | Test vessels were covered with a plastic film which had an opening for aeration. |
| **Photoperiod**<br>16 hours light, 8 hours dark<br>(Light intensity 500 to 1000 lux) | 16 hours light, 8 hours dark<br>Light intensity 800 to 1200 lux |
| **Food**<br>Green algae (e.g., *Scenedesmus subspicatus, Chlorella vulgaris*) or flaked fish food as a ground powder, suspension, or filtrate | Tetra Min® suspension, 1 g/40 mL |

| Guideline Criteria | Reported Information |
|---|---|
| **Food Concentration and Frequency**<br>Preferably feed daily but at least 3 times per week.<br>day 1 to 10: 0.25-0.5 mg per larvae per day<br>remainder of test: 0.5-1 mg per larvae per day (keep to a minimum, should not accumulate on sediment surface, cause overlying water to be cloudy or cause drop in DO) | Every 1 to 2 days<br><br>Days -1 to 17: 0.5 to 2 mL per vessel<br><br>After Day 17, no further feeding was performed due to algae growth. |

**C. Test Design**

| Guideline Criteria | Reported Information |
|---|---|
| **Duration**<br>*Chironomus riparius*: 28 days (if midges emerge early the test can be terminated after a minimum of 5 days after emergence of the last adult in the control). | 28 days |
| **Nominal Concentrations**<br>Negative control, solvent control (if a solvent was used) and at least 5 test concentrations. (Note exception to dilution factors described below can be made for shallow slope responses but minimum number of test concentrations may need to be increased)<br><br>ECx endpoint: test concentrations should bracket ECx and span the environmental concentration range. Dilution factor should not be greater than two between exposure concentrations.<br><br>NOEC/LOEC endpoint: factor between concentrations must not be greater than 3. | Negative control, solvent control, 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 mg/L<br><br>ECx endpoint: test concentrations were expected to bracket the $EC_0$ to $EC_{100}$ range. The dilution factor was 2.<br><br>NOAEC/LOAEC endpoint: (same) |

| Guideline Criteria | Reported Information |
|---|---|
| **Number of Test Organisms**** | |
| ECx endpoint: 60 larvae per treatment level; 3 replicates per treatment level | ECx endpoint: 75 larvae per treatment level; 3 replicates per treatment level, with 25 larvae per replicate |
| NOAEC/LOAEC endpoint: at least 80 larvae per treatment level with at least 4 replicates per treatment level (adequate power to detect a 20% difference, Type I error rate 5%) | NOAEC/LOAEC endpoint: (same) |
| *(Optional) If data on 10-day growth and survival are needed additional replicates (number based on ECx or NOEC/LOEC endpoint determination) should be included at test initiation.. | *(Optional) 10-day growth data were not collected. |
| **Test organisms randomly or impartially assigned to test vessels?** | Yes |
| **Overlying Water Parameter Measurements** | |
| 1. Dissolved oxygen should be measured daily in all test chambers. | 1. – 3. Temperature, pH, and dissolved oxygen were measured in each vessel on Days -1, 6, 14, 20, and 27. |
| 2. Temperature and pH should be measured in all test chambers at the start and end of the test and at least once a week during the test. | |
| 3. Temperature should be monitored at least hourly throughout the test in one test chamber. | |
| 4. Hardness and ammonia should be measured in the controls and one test chamber at the highest concentration at the start and end of the test. | 4. Not determined |

| Guideline Criteria | Reported Information |
|---|---|
| **Chemical Analysis-Overlying Water**<br>At a minimum must be analyzed at test initiation (i.e., one hour after introduction of test substance into the test chamber) and at the end of the test in at least the highest concentration and one lower concentration. | Overlying water was sampled from the biological test vessels prepared at 1 and 8 mg/L on Days 0 (2 hours after application) and 28. Overlying water was sampled from biological vessels prepared at all levels on Day 3.<br><br>All aqueous samples were analyzed for trifluralin by direct injection into an HPLC system with UV (275 nm) detection. |
| **Interstitial Water and Sediment Isolation Method**<br>Centrifugation (e.g., 10,000 g and 4 EC for 30 min) is recommended. If test substance is demonstrated not to adsorb to filters, filtration may be acceptable. | Sediment and pore water were isolated using vacuum filtration. |
| **Chemical Analysis-Interstitial Water**<br>At a minimum must be analyzed at the end of the test in at least the highest concentration and one lower concentration. | Not assessed. |
| **Chemical Analysis-Bulk Sediment**<br>At a minimum must be analyzed at the end of the test in at least the highest concentration and one lower concentration. | The sediment of surrogate vessels prepared at 1 and 8 mg/L were collected for analysis on Days 0, 7, and 28. Sediment was extracted with acetone, and extracts were analyzed for trifluralin using HPLC with UV (275 nm) detection. |

## 12. REPORTED RESULTS

### A. General Results

| Guideline Criteria | Reported Information |
|---|---|
| **Quality assurance and GLP compliance statements were included in the report?** | Yes. This study was conducted in compliance with the GLP standards of the OECD and U.S. EPA. |
| **Control Mortality**<br><30% | Yes |
| **Did chironomids emerge in controls between day 12 and 23?** | Negative control – days 11 to 21<br>Solvent control – days 11 to 17 |
| **Control Emergence**<br>Mean emergence between 50-70% | Negative control – 97.3% emergence<br>Solvent control – 94.9% emergence |
| **Data Endpoints**<br>Emergence Test (28 day)<br>- Number alive<br>- Time to emergence<br>- Number of emerged male and female midges<br>- Number of visible pupae that have failed to emerge<br>- Number of egg masses deposited<br>- Observations of other effects, abnormal behavior, or appearance or clinical signs (e.g., leaving sediment, unusual swimming)<br><br>Growth and Survival (10-day) (Optional)<br>- Number alive<br>- Instar level of surviving larvae<br>- Dry weight (ash free) per test chamber of surviving larvae by instar level | Emergence Test (28 days)<br>- Number alive<br>- Time to emergence<br>- Number of emerged male and female midges<br><br>Growth and Survival (10-day) (Optional)<br>N/A |
| **Raw data included?** | Yes |

**Effects Data**

Table 1. Summary of trifluralin effects on *Chironomus riparius* emergence success and sex ratio

| Toxicant Concentration | | | | Initial No. | Mean Number Emerged | | | Mean Sex Ratio[b] (%) | |
|---|---|---|---|---|---|---|---|---|---|
| Nominal Overlying Water (mg ai/L) | Mean Measured TWA[a] | | | | ♂ | ♀ | Total | $ER_{♂}$ | $ER_{♀}$ |
| | Overlying Water (mg ai/L) | Sediment (mg ai/vessel) | Pore Water (mg ai/L) | | | | | | |
| Negative control | Not calculable | Not assessed | Not assessed | 75 | 33 | 40 | 73 | 45 | 55 |
| Solvent control | Not assessed | Not assessed | Not assessed | 78 | 31 | 43 | 74 | 42 | 58 |
| 0.25 | Not calculable | Not assessed | Not assessed | 75 | 33 | 37 | 70 | 47 | 53 |
| 0.5 | Not calculable | Not assessed | Not assessed | 76 | 31 | 42 | 73 | 42 | 58 |
| 1.0 | 0.0497 | | Not assessed | 76 | 40 | 32 | 72 | 56 | 44 |
| 2.0 | Not calculable | Not assessed | Not assessed | 75 | 39 | 24 | 63 | 62 | 38 |
| 4.0 | Not calculable | Not assessed | Not assessed | 75 | 22 | 23 | 45 | 49 | 51 |
| 8.0 | 0.0495 | | Not assessed | 75 | 14 | 16 | 30 | 47 | 53 |

[a] Reviewer-calculated time-weighted averages (refer to associated Excel spreadsheet); results were rounded to three significant figures. For overlying water, the LOD and LOQ were 0.035 and 0.074 mg ai/L, respectively. For sediment samples, the LOD and LOQ were 0.153 and 0.230 mg/vessel, respectively.

[b] $ER_{♂}$ = number of emerged males/number of emerged larvae x 100; $ER_{♀}$ = number of emerged females/number of emerged larvae x 100; reviewer-calculated.

Table 2. Summary of trifluralin effects on *Chironomus riparius* development time and rate.

| Toxicant Concentration | | | | | Arc-sine Transformed Emergence Rate[b] | Mean Development Rate[b][c] (1/day) |
|---|---|---|---|---|---|---|
| Overlying Water (mg ai/L) | | Mean Measured TWA[a] | | | | |
| Nominal | Measured on day 3 | Overlying Water (mg ai/L) | Sediment (mg ai/vessel) | Pore Water (mg ai/L) | | |
| Negative control | <LOD | Not calculable[d] | Not assessed | Not assessed | 1.48 | 0.0837 |
| Solvent control | Not assessed | Not assessed | Not assessed | Not assessed | 1.43 | 0.0793 |
| 0.25 | 0.042 | Not calculable | Not assessed | Not assessed | 1.36 | 0.0783 |
| 0.5 | 0.047 | Not calculable | Not assessed | Not assessed | 1.41 | 0.0729* |
| 1.0 | 0.107 | 0.0497 | 0.1986[e] | Not assessed | 1.43 | 0.0738* |
| 2.0 | 0.058 | Not calculable | Not assessed | Not assessed | 1.18 | 0.0692* |
| 4.0 | 0.297 | Not calculable | Not assessed | Not assessed | 0.898* | 0.0631* |
| 8.0 | 0.164 | 0.0495 | 7.1584[e] | Not assessed | 0.684* | 0.0500* |

* Significantly difference compared to the solvent control at $p<0.05$.

[a] Reviewer-calculated time-weighted averages (refer to associated Excel spreadsheet); results were rounded to three significant figures. For overlying water, the LOD and LOQ were 0.035 and 0.074 mg ai/L, respectively. For sediment samples, the LOD and LOQ were 0.153 and 0.230 mg/vessel, respectively.

[b] Means were reviewer-calculated using replicate data provided in the study report (refer to associated Excel spreadsheet); results were rounded to three significant figures.

[c] Mean development rate $= \sum_{i=1}^{m} \frac{f_i x_i}{n_e}$ where: $i$ = index of inspection interval; m = maximum number of inspection intervals; $f_i$ = number of midges emerged in the inspection interval $i$; $n_e$ = total number of midges emerged; and $x_i = 1 / \left( day_i - \frac{l_i}{2} \right)$ which is the development rate of the midges emerged in interval $i$; $day_i$ = inspection day (days since application); and $l_i$ = length of inspection interval $i$ (days, 1 day in this study).

[d] Vessels were only measured at one time point.

[e] Nominal concentrations are 1.65 mg/vessel and 13.2 mg/vessel, respectively.

Toxicity Observations (in terms of nominal concentrations): Reviewer-calculated emergence rates were 97.3, 94.9, 93.3, 96.0, 94.7, 84.0, 60.0, and 40.0% for the negative control, solvent control, 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 mg ai/L levels, respectively. Corresponding arcsine transformed emergence rates were 1.48, 1.43, 1.36, 1.41, 1.43, 1.18, 0.898, and 0.684, respectively. Differences were statistically different ($p<0.05$) compared to the solvent control at the 4.0 and 8.0 mg ai/L levels. The NOAEC for emergence was 2.0 mg ai/L. The following ECx values for emergence were calculated by the study author:

Table 3. $EC_x$ values for midge emergence and associated 95% confidence limits, mg/L.

| $EC_x$ | | Lower C.I. | Upper C.I. |
|---|---|---|---|
| $EC_1$ | 0.17 | 0.09 | 0.33 |
| $EC_5$ | 0.51 | 0.33 | 0.77 |
| $EC_{10}$ | 0.89 | 0.65 | 1.23 |
| $EC_{15}$ | 1.31 | 1.00 | 1.71 |
| $EC_{20}$ | 1.77 | 1.40 | 2.25 |
| **$EC_{50}$** | **6.60** | **4.82** | **9.06** |
| $EC_{80}$ | 24.59 | 14.01 | 43.14 |

Development rate was the most sensitive endpoint, and averaged 0.0837, 0.0793, 0.0783, 0.0729, 0.0738, 0.0692, 0.0631, and 0.0500 $days^{-1}$ for the negative control, solvent control, 0.25, 0.50, 1.0, 2.0, 4.0, and 8.0 mg ai/L levels, respectively. Differences were statistically different ($p<0.05$) compared to the solvent control at the $\geq$0.50 mg ai/L levels. The NOAEC for development rate was 0.25 mg ai/L.

**B. Statistical Results (From Study Report)**

$EC_x$ values with associated 95% confidence intervals were determined using probit analysis, or in case of failure, non-parametric methods such as moving averages or simple interpolation. Calculations were determined with the EASY ASSAY Critical Values computer program (Ver. 3.0).

In addition, the number of emerged midges, emergence rate, and development rate (combined genders) were subjected to statistical analysis on a per vessel (or replicate) basis, and emergence rates were arcsine-transformed prior to analysis. Data were assessed for homogeneity of variance using the Kruskal-Wallis (emerged midges) or Bartlett's (emergence and development rates) test. In all cases, the homogeneity hypothesis was accepted, and that NOAEC/LOAEC values were determined using ANOVA and Dunnett and Williams multiple t-tests at the $p=0.05$ level.

Most sensitive endpoint: development rate

| Endpoint | Methods | $EC_{50}$ (95% CI) (mg/L) | NOAEC (mg/L) | LOAEC (mg/L) |
|---|---|---|---|---|
| 28-d No. Emerged Midges | Dunnett Williams | 6.60 (4.82 to 9.06) | 2.0 | 4.0 |
| 28-d Emergence Rate | Dunnett Williams | --- | 2.0 | 4.0 |
| 28-d Development Rate | Dunnett Williams | --- | 0.25 | 0.50 |
| 10-d Survival (Optional) | --- | --- | --- | --- |
| 10-d Growth (Optional) | --- | --- | --- | --- |

## 13. VERIFICATION OF STATISTICAL RESULTS

Analyses were performed using TOXSTAT 3.5 and Nuthatch statistical software with nominal overlying water concentrations. Endpoints that were statistically analyzed included emergence percentages and development rates.

Negative and solvent control data were compared using a t-test (alpha = 0.05); for emergence and development rates, no significant differences were observed. All comparisons of treatment groups were made to the negative control.

Data were tested for normality using the Shapiro-Wilk's test, and for homogeneity of variance using Bartlett's test. Percent emergence data were transformed using the arcsin-square root transformation to meet assumptions. After transformation, all data were both normal with homogenous variance; a parametric analysis was conducted using Williams' test (alpha = 0.05).

The $EC_{50}$ for emergence percentage was calculated using the Bruce and Versteeg approach in the Nuthatch software. $IC_{50}$ for development rate was not calculated as a 50% decrease from the control was not observed. $IC_{50}$ for development was visually determined at > 8.0 mg/L.

**Summary of Statistical Methods used for NOAEC/LOAEC Analyses.**

| Endpoint | Solvent vs Dilution Control | | NOAEC/LOAEC | |
|---|---|---|---|---|
| | Method | Diff [1] (%) | Method | Diff [2] (%) |
| 28-d Emergence Rate | Student's t-test | 2.5 | ANOVA<br>Williams' | 13.7 |
| 28-d Development Rate | Student's t-test | 5.3 | ANOVA<br>Williams' | 6.5 |
| 10-d Survival (Optional) | --- | --- | --- | --- |
| 10-day Dry Weight (Optional) | --- | --- | --- | --- |

[1] Difference between the mean dilution water and solvent control responses.
[2] Difference between the dilution water and NOAEC concentration treatment.

Most sensitive endpoint: Development rate

**Verification Statistical Endpoint Values[a].**

| Statistical Endpoint | 28-day Emergence | 28-day Development Rate | 10-d Survival | 10-d Dry Weight |
|---|---|---|---|---|
| NOAEC | 2 mg/L | 0.25 mg/L | --- | --- |
| LOAEC | 4 mg/L | 0.5 mg/L | --- | --- |
| $EC_{50}/IC_{50}$ (95% C.I.) | 6.9 mg/L (4.6 to 10 mg/L) | >8.0 mg/L | --- | --- |
| Slope (Standard Error) | 1.71±0.535 | N/A | --- | --- |

[a] Results are based on nominal overlying water concentrations.

**14. REVIEWER'S COMMENTS:**

The reviewer's conclusions generally agreed with the study author's. The NOAEC values, calculated by the reviewer using the negative control as a comparison to treated groups, were identical to those calculated by the study authors (using the solvent control as a comparison to treated groups). Results calculated by the reviewer will be reported in the study conclusions.

This study does not fulfill any current U.S. EPA guideline. However, it closely followed methods provided in OECD Guideline 219 (April 2004), "Sediment-Water Chironomid Toxicity Test Using Spiked Water", with the primary objective being to determine the median effect concentrations ($EC_x$) associated with emergence (i.e., survival) of *Chironomus riparius*. In order for the test to be valid, OECD Guidance requires the following conditions: The emergence in the controls must be at least 70% at the end of the test; *C. riparius* emergence to adults should occur between 12 and 23 days after their insertion into the vessels; at the end of the test, pH and the dissolved oxygen concentration should be measured in each vessel (the oxygen concentration should be at least 60% of the air saturation value at the temperature used, the pH of overlying water should be in the 6-9 range in all test vessels); and the water temperature should not differ by more than ±1.0°C. In this study, the pH values increased from an average of 7.93 on Day -1 to 9.99 on Day 27, and exceeded the limits of the proposal guideline due to the algae growth (which was also indicated by the high oxygen concentrations). The study author reported that this did not negatively affect the organisms in the test. All other validity requirements were fulfilled.

Overlying water (volume not reported) was sampled directly from the biological vessels prepared on Day 3 (all levels) and Days 0, 7, and 28 (1.0 and 8.0 mg/L levels only). In addition, surrogate vessels were prepared and used for sediment analysis on Days 0, 7, and 28 (1.0 and 8.0 mg/L levels only). Although it was reported that overlying water was analyzed in surrogate test vessels collected on Days 0, 7, and 28, the data provided for Days 0 and 7 (once converted from mg/vessel to mg/L) were identical to data obtained from analysis of overlying water collected from biological samples for Days 0 and 3. Therefore, it was apparent that only sediment from the surrogate vessels was analyzed.

The volume of overlying water removed (directly from the biological samples) for trifluralin analysis was not reported; therefore, it is unknown what, if any, affect the change in volume had on the biological load or concentration of test substance in the system.

The study was conducted for 30 days, 9 days following emergence of the last adult and after 90% of the chironomids had emerged from the solvent control vessels. However, for evaluation of the study, data from only 28 days were taken into account.

When possible, TWA concentrations were calculated by the reviewer using the following equation (refer to associated Excel worksheet in Appendix II):

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:
C TWA is the time-weighted average concentration,
C j is the concentration measured at time interval j (j = 0, 1, 2,...n)
t j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j (e.g., t 0 = 0 hours (test initiation), t 1 =24 hours, t 2 =96 hours).

The definitive study was conducted from May 3 to June 2, 1996.

**15. REFERENCES:**

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## APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL ANALYSIS:

arcsin-transformed emergence rate
File: 7013e Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

| INTERVAL | <-1.5 | -1.5 to <-0.5 | -0.5 to 0.5 | >0.5 to 1.5 | >1.5 |
|---|---|---|---|---|---|
| EXPECTED | 1.407 | 5.082 | 8.022 | 5.082 | 1.407 |
| OBSERVED | 0 | 7 | 4 | 10 | 0 |

Calculated Chi-Square goodness of fit test statistic = 10.3137
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

arcsin-transformed emergence rate
File: 7013e Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.655

W = 0.961

Critical W (P = 0.05) (n = 21) = 0.908
Critical W (P = 0.01) (n = 21) = 0.873

Data PASS normality test at P=0.01 level. Continue analysis.

arcsin-transformed emergence rate
File: 7013e Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 90.89
Closest, conservative, Table H statistic = 1705.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 2
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 2.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal

but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

arcsin-transformed emergence rate
File: 7013e Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

---

Calculated B statistic = 6.55
Table Chi-square value = 16.81 (alpha = 0.01)
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00
Used for Chi-square table value ==> df (#groups-1) = 6

---

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

arcsin-transformed emergence rate
File: 7013e Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

---

| | | | |
|---|---|---|---|
| GRP1 (SOLVENT CRTL) MEAN = | 1.4752 | CALCULATED t VALUE = | 0.2487 |
| GRP2 (BLANK CRTL) MEAN = | 1.4336 | DEGREES OF FREEDOM = | 4 |
| DIFFERENCE IN MEANS = | 0.0416 | | |

---

TABLE t VALUE (0.05 (2), 4) = 2.776 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 4) = 4.604 NO significant difference at alpha=0.01

arcsin-transformed emergence rate
File: 7013e Transform: NO TRANSFORMATION

ANOVA TABLE

---

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 6 | 1.667 | 0.278 | 5.915 |
| Within (Error) | 14 | 0.655 | 0.047 | |

---

```
Total                20                2.321
--------------------------------------------------------------------------------

  Critical F value =   2.85  (0.05,6,14)
  Since  F > Critical F  REJECT  Ho:All groups equal


arcsin-transformed emergence rate
File: 7013e          Transform: NO TRANSFORMATION

      DUNNETTS TEST   -   TABLE 1 OF 2              Ho:Control<Treatment
--------------------------------------------------------------------------------
                                TRANSFORMED     MEAN CALCULATED IN
GROUP    IDENTIFICATION           MEAN          ORIGINAL UNITS     T STAT  SIG
----- --------------------     ----------     ------------------   ------  ---
  1            neg control        1.475              1.475
  2                   0.25        1.357              1.357          0.666
  3                    0.5        1.408              1.408          0.379
  4                      1        1.434              1.434          0.235
  5                      2        1.179              1.179          1.672
  6                      4        0.898              0.898          3.261  *
  7                      8        0.684              0.684          4.467  *
--------------------------------------------------------------------------------
Dunnett table value =  2.53      (1 Tailed Value, P=0.05,   df=14,6)


arcsin-transformed emergence rate
File: 7013e          Transform: NO TRANSFORMATION

      DUNNETTS TEST   -   TABLE 2 OF 2              Ho:Control<Treatment
--------------------------------------------------------------------------------
                              NUM OF   Minimum Sig Diff  % of      DIFFERENCE
GROUP    IDENTIFICATION       REPS     (IN ORIG. UNITS)  CONTROL   FROM CONTROL
----- --------------------   -------  ----------------  -------  ------------
  1            neg control      3
  2                   0.25      3             0.448       30.4         0.118
  3                    0.5      3             0.448       30.4         0.067
  4                      1      3             0.448       30.4         0.042
  5                      2      3             0.448       30.4         0.296
  6                      4      3             0.448       30.4         0.577
  7                      8      3             0.448       30.4         0.791
--------------------------------------------------------------------------------


arcsin-transformed emergence rate
File: 7013e          Transform: NO TRANSFORMATION

        WILLIAMS TEST  (Isotonic regression model)    TABLE 1 OF 2
--------------------------------------------------------------------------------
GROUP                                    ORIGINAL      TRANSFORMED    ISOTONIZED
         IDENTIFICATION          N       MEAN            MEAN          MEAN
------  --------------------    ---   ----------     ----------     ----------
   1             neg control    3       1.475          1.475          1.475
```

| | | | | | |
|---|---|---|---|---|---|
| 2 | 0.25 | 3 | 1.357 | 1.357 | 1.400 |
| 3 | 0.5 | 3 | 1.408 | 1.408 | 1.400 |
| 4 | 1 | 3 | 1.434 | 1.434 | 1.400 |
| 5 | 2 | 3 | 1.179 | 1.179 | 1.179 |
| 6 | 4 | 3 | 0.898 | 0.898 | 0.898 |
| 7 | 8 | 3 | 0.684 | 0.684 | 0.684 |

arcsin-transformed emergence rate
File: 7013e Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
|---|---|---|---|---|---|
| neg control | 1.475 | | | | |
| 0.25 | 1.400 | 0.428 | | 1.76 | k= 1, v=14 |
| 0.5 | 1.400 | 0.428 | | 1.85 | k= 2, v=14 |
| 1 | 1.400 | 0.428 | | 1.88 | k= 3, v=14 |
| 2 | 1.179 | 1.677 | | 1.89 | k= 4, v=14 |
| 4 | 0.898 | 3.269 | * | 1.90 | k= 5, v=14 |
| 8 | 0.684 | 4.479 | * | 1.91 | k= 6, v=14 |

s = 0.216
Note: df used for table values are approximate when v > 20.

7013E : arcsin-transformed emergence rate

Williams Test

[One-Sided Test for Decrease, alpha = 0.050000 ]

| Dose | Isotone Means | T-bar | P-value | Significance |
|---|---|---|---|---|
| 0 | 1.48 | . | | |
| 0.25 | 1.4 | 0.4278 | N.S. | |
| 0.5 | 1.4 | 0.4278 | N.S. | |
| 1 | 1.4 | 0.4278 | N.S. | |
| 2 | 1.18 | 1.676 | N.S. | |
| 4 | 0.898 | 3.269 | <0.005 | * |
| 8 | 0.684 | 4.478 | <0.005 | * |

"*"=Significant; "N.S."=Not Significant.

Estimates of EC%

| Parameter | Estimate | 95% Bounds Lower | 95% Bounds Upper | Std.Err. | Lower Bound /Estimate |
|---|---|---|---|---|---|
| EC5 | 0.75 | 0.17 | 3.4 | 0.31 | 0.22 |
| EC10 | 1.2 | 0.37 | 4.0 | 0.25 | 0.30 |

| | | | | | |
|---|---|---|---|---|---|
| EC25 | 2.8 | 1.4 | 5.6 | 0.15 | 0.49 |
| EC50 | 6.9 | 4.6 | 10. | 0.084 | 0.67 |

Slope = 1.71 Std.Err. = 0.535

Goodness of fit: p = 0.88 based on DF= 4.0 14.

---

7013E : arcsin-transformed emergence rate

---

Observed vs. Predicted Treatment Group Means

---

| Dose | #Reps. | Obs. Mean | Pred. Mean | Obs. -Pred. | Pred. %Control | %Change |
|---|---|---|---|---|---|---|
| 0.00 | 3.00 | 1.48 | 1.45 | 0.0274 | 100. | 0.00 |
| 0.250 | 3.00 | 1.36 | 1.44 | -0.0805 | 99.3 | 0.695 |
| 0.500 | 3.00 | 1.41 | 1.41 | -0.00240 | 97.4 | 2.58 |
| 1.00 | 3.00 | 1.43 | 1.34 | 0.0958 | 92.4 | 7.60 |
| 2.00 | 3.00 | 1.18 | 1.19 | -0.00941 | 82.1 | 17.9 |
| 4.00 | 3.00 | 0.898 | 0.952 | -0.0538 | 65.7 | 34.3 |
| 8.00 | 3.00 | 0.684 | 0.661 | 0.0230 | 45.7 | 54.3 |

development rate
File: 7013d Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

---

| INTERVAL | <-1.5 | -1.5 to <-0.5 | -0.5 to 0.5 | >0.5 to 1.5 | >1.5 |
|---|---|---|---|---|---|
| EXPECTED | 1.407 | 5.082 | 8.022 | 5.082 | 1.407 |
| OBSERVED | 0 | 7 | 7 | 7 | 0 |

---

Calculated Chi-Square goodness of fit test statistic = 4.3919
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

development rate
File: 7013d Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

---

D = 2.690

W = 0.977

Critical W (P = 0.05) (n = 21) = 0.908
Critical W (P = 0.01) (n = 21) = 0.873

```
------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.


development rate
File: 7013d        Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =    9.43
Closest, conservative, Table H statistic = 1705.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =   7,    df (# reps-1) =       2
Actual values    ==>     R (# groups) =   7,    df (# avg reps-1) =   2.00

------------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).


development rate
File: 7013d       Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
------------------------------------------------------------------------------

Calculated B statistic =    3.24
Table Chi-square value =   16.81  (alpha = 0.01)
Table Chi-square value =   12.59  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.00
Used for Chi-square table value ==>   df (#groups-1) =   6
------------------------------------------------------------------------------

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).


development rate
File: 7013d        Transform: NO TRANSFORM

       t-test of Solvent and Blank Controls          Ho:GRP1 MEAN = GRP2 MEAN
------------------------------------------------------------------------------
-
 GRP1 (SOLVENT CRTL) MEAN =     8.3749     CALCULATED t VALUE =     1.2095
```

```
 GRP2 (BLANK CRTL) MEAN    =      7.9307     DEGREES OF FREEDOM =      4
 DIFFERENCE IN MEANS       =      0.4442
-----------------------------------------------------------------------------
-
TABLE t VALUE (0.05 (2), 4) =  2.776    NO significant difference at
alpha=0.05
TABLE t VALUE (0.01 (2), 4) =  4.604    NO significant difference at
alpha=0.01


development rate
File: 7013d         Transform: NO TRANSFORMATION

                                ANOVA TABLE
-----------------------------------------------------------------------------

SOURCE              DF              SS                MS              F
-----------------------------------------------------------------------------
Between              6              21.846            3.641          18.964

Within (Error)      14               2.690            0.192
-----------------------------------------------------------------------------
Total               20              24.536
-----------------------------------------------------------------------------

  Critical F value =   2.85  (0.05,6,14)
  Since  F > Critical F  REJECT  Ho:All groups equal


development rate
File: 7013d         Transform: NO TRANSFORMATION

      DUNNETTS TEST   -   TABLE 1 OF 2            Ho:Control<Treatment
-----------------------------------------------------------------------------

                              TRANSFORMED    MEAN CALCULATED IN
GROUP    IDENTIFICATION          MEAN          ORIGINAL UNITS    T STAT  SIG
----- --------------------    ----------    ------------------  ------  ---
  1            neg control       8.375             8.375
  2                   0.25       7.829             7.829          1.526
  3                    0.5       7.287             7.287          3.041  *
  4                      1       7.382             7.382          2.775  *
  5                      2       6.925             6.925          4.054  *
  6                      4       6.305             6.305          5.786  *
  7                      8       5.003             5.003          9.425  *
-----------------------------------------------------------------------------
Dunnett table value =  2.53     (1 Tailed Value, P=0.05,  df=14,6)


development rate
File: 7013d         Transform: NO TRANSFORMATION

      DUNNETTS TEST   -   TABLE 2 OF 2            Ho:Control<Treatment
-----------------------------------------------------------------------------
```

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | neg control | 3 | | | |
| 2 | 0.25 | 3 | 0.905 | 10.8 | 0.546 |
| 3 | 0.5 | 3 | 0.905 | 10.8 | 1.088 |
| 4 | 1 | 3 | 0.905 | 10.8 | 0.993 |
| 5 | 2 | 3 | 0.905 | 10.8 | 1.450 |
| 6 | 4 | 3 | 0.905 | 10.8 | 2.070 |
| 7 | 8 | 3 | 0.905 | 10.8 | 3.372 |

development rate
File: 7013d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
|---|---|---|---|---|---|
| 1 | neg control | 3 | 8.375 | 8.375 | 8.375 |
| 2 | 0.25 | 3 | 7.829 | 7.829 | 7.829 |
| 3 | 0.5 | 3 | 7.287 | 7.287 | 7.335 |
| 4 | 1 | 3 | 7.382 | 7.382 | 7.335 |
| 5 | 2 | 3 | 6.925 | 6.925 | 6.925 |
| 6 | 4 | 3 | 6.305 | 6.305 | 6.305 |
| 7 | 8 | 3 | 5.003 | 5.003 | 5.003 |

development rate
File: 7013d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
|---|---|---|---|---|---|
| neg control | 8.375 | | | | |
| 0.25 | 7.829 | 1.525 | | 1.76 | k= 1, v=14 |
| 0.5 | 7.335 | 2.907 | * | 1.85 | k= 2, v=14 |
| 1 | 7.335 | 2.907 | * | 1.88 | k= 3, v=14 |
| 2 | 6.925 | 4.052 | * | 1.89 | k= 4, v=14 |
| 4 | 6.305 | 5.784 | * | 1.90 | k= 5, v=14 |
| 8 | 5.003 | 9.422 | * | 1.91 | k= 6, v=14 |

s = 0.438
Note: df used for table values are approximate when v > 20.

**APPENDIX II: COPY OF REVIEWER'S TWA CALCULATIONS (USING EXCEL):**

OVERLYING WATER

| Nominal Conc. mg ai/L | Time (Day) | Measured Conc. (mg ai/L) Biological Vessels | TWA (mg ai/L) |
|---|---|---|---|
| 1 | 0 | 0.409 | |
| | 3 | 0.107 | |
| | 7 | 0.0175 | |
| | 28 | 0.0175 | |
| | | | **0.04966** |
| 8 | 0 | 0.273 | |
| | 3 | 0.164 | |
| | 7 | 0.0175 | |
| | 28 | 0.0175 | |
| | | | **0.04950** |

SEDIMENT

| Nominal Conc. mg ai/L | Time (Day) | Measured Conc. (mg ai/vessel) Surrogate Vessels | TWA (mg ai/vessel) |
|---|---|---|---|
| 1 =1.65 mg ai/vessel | 0 | 0.327 | |
| | 7 | 0.258 | |
| | 28 | 0.0765 | |
| | | | **0.19856** |
| 8 =13.2 mg ai/vessel | 0 | 7.771 | |
| | 7 | 8.27 | |
| | 28 | 5.472 | |
| | | | **7.15838** |

When necessary, half of the LOD was used for calculation purposes.

**APPENDIX III: COPY OF REVIEWER'S MEAN EMERGENCE RATE AND DEVELOPMENT RATE CALCULATIONS (USING EXCEL):**

MEAN EMERGENCE RATE

| Nominal Conc. | ARCsin-transformed emergence rate | | | |
|---|---|---|---|---|
| mg/L | Rep. 1 | Rep. 2 | Rep. 3 | Mean |
| Neg. control | 1.28404 | 1.5708 | 1.5708 | 1.48 |
| Sol. Control | 1.5708 | 1.15928 | 1.5708 | 1.43 |
| 0.25 | 1.5708 | 1.21705 | 1.28404 | 1.36 |
| 0.5 | 1.28404 | 1.36944 | 1.5708 | 1.41 |
| 1 | 1.15928 | 1.5708 | 1.5708 | 1.43 |
| 2 | 0.96953 | 1.28404 | 1.28404 | 1.18 |
| 4 | 1.0132 | 1.21705 | 0.46365 | 0.898 |
| 8 | 0.72525 | 0.6435 | 0.68472 | 0.684 |

MEAN DEVELOPMENT RATE

| Nominal Conc. | | | | |
|---|---|---|---|---|
| mg/L | Rep. 1 | Rep. 2 | Rep. 3 | Mean |
| Neg. control | 0.083182 | 0.086522 | 0.081544 | 0.0837 |
| Sol. Control | 0.081632 | 0.08362 | 0.072669 | 0.0793 |
| 0.25 | 0.078707 | 0.083128 | 0.073035 | 0.0783 |
| 0.5 | 0.068927 | 0.075501 | 0.074182 | 0.0729 |
| 1 | 0.066085 | 0.079777 | 0.075599 | 0.0738 |
| 2 | 0.065704 | 0.072292 | 0.069744 | 0.0692 |
| 4 | 0.064968 | 0.060521 | 0.063661 | 0.0631 |
| 8 | 0.055409 | 0.045562 | 0.049112 | 0.0500 |